Exhibit 1

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Anti-allodynic efficacy of the χ-conopeptide, Xen2174, in rats with neuropathic pain

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Abstract

Xen2174 is a structural analogue of Mr1A, a χ-conopeptide recently isolated from the venom of the marine cone snail, *Conus marmoreus*. Although both χ-conopeptides are highly selective inhibitors of the norepinephrine transporter (NET), Xen2174 has superior chemical stability relative to Mr1A. It is well-known that tricyclic antidepressants (TCAs) are also potent NET inhibitors, but their poor selectivity relative to other monoamine transporters and various G-protein-coupled receptors, results in dose-limiting side-effects in vivo. As TCAs and the α_2-adrenoceptor agonist, clonidine, have established efficacy for the relief of neuropathic pain, this study examined whether intrathecal (i.t.) Xen2174 alleviated mechanical allodynia in rats with either a chronic constriction injury of the sciatic nerve (CCI-rats) or an L5/L6 spinal-nerve injury. The anti-allodynic responses of i.t. Mr1A and i.t. morphine were also investigated in CCI-rats. Paw withdrawal thresholds were assessed using calibrated von Frey filaments. Bolus doses of i.t. Xen2174 produced dose-dependent relief of mechanical allodynia in CCI-rats and in spinal nerve-ligated rats. Dose-dependent anti-allodynic effects were also produced by i.t. bolus doses of Mr1A and morphine in CCI-rats, but a pronounced 'ceiling' effect was observed for i.t. morphine. The side-effect profiles were mild for both χ-conopeptides with an absence of sedation. Confirming the noradrenergic mechanism of action, i.t. co-administration of yohimbine (100 nmol) with Xen2174 (10 nmol) abolished Xen2174 s anti-allodynic actions. Xen2174 appears to be a promising candidate for development as a novel therapeutic for i.t. administration to patients with persistent neuropathic pain.

Keywords: X-conopeptides: intrathecal dosing; Rat model of neuropathic pain: chronic constriction injury (CCI); L5/L6 nerve ligation; Tactile allodynia; Von Frey filaments; Morphine

1. Introduction

Neuropathic pain is a persistent, pathological condition that may develop following nerve injury secondary to trauma, inflammation or degenerative disease in the peripheral and/or the central nervous system (CNS) (Bridges et al., 2001; Woolf and Mannion, 1999) and has been reported to be a significant component of chronic cancer pain (Davis and Walsh, 2004; Strömgren et al., 2004). Tricyclic anti-depressants notably those with preferences for the norepinephrine transporter (NET) (e.g. amitriptyline) have been shown to have efficacy in both human clinical pain and experimental models of nerve injury pain (Collins et al., 2000; Fishbain et al., 2000; McQuay, Tramer, Nue, Carroll, Wiffen and Moore, 1996). The activity of these NET inhibiting agents after spinal delivery is accordingly believed to reflect the intrinsic regulatory activity of bulbospinal noradrenergic projections into the spinal dorsal horn (Tyce and Yaksh, 1981). Consistent with this role of spinal norepinephrine in regulating spinal pain processing in general and neuropathic pain in particular, spinally delivered α_2-adrenoceptor agonists such as clonidine, have been

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shown to be efficacious in a variety of neuropathic pain states in preclinical models (Abelson and Hoglund, 2004; Duflo, Li, Bantel, Pancaro, Vincler and Eisenach, 2002; Fairbanks et al., 2000; Hao et al., 1999; Kawamata, Omote, Yamamoto, Toriyabe, Wada and Namiki, 2003; Paqueron et al., 2003; Yaksh et al., 1995) as well as in human pain states including those arising from cancer (Chong and Bajwa, 2003; Eisenach et al., 1996; Hassenbusch et al., 2002).

Specification of the unique importance of these endogenous bulbospinal noradrenergic projections in the anti-hyperpathic actions remains controversial because of the relatively poor specificity of common NET inhibitors, and in particular the TCAs for NET, relative to dopamine and serotonin transporters (DAT and SERT respectively) as well as to some CNS receptors in vivo (e.g. muscarinic acetylcholine receptors) (Bryan-Lluka et al., 2003).

Recently, Mr1A, the first χ-conopeptide to be isolated and identified from the crude venom of *Conus marmoreus*, a cone snail found in the waters of the Great Barrier Reef (Sharpe et al., 2001, 2003), has been shown to produce antinociception after i.t. administration in mice (McIntosh et al., 2000). Mr1A has been shown to be a selective and reversible inhibitor of neuronal NET (Bryan-Lluka et al., 2003; Sharpe et al., 2001, 2003). Interestingly, Mr1A non-competitively inhibits [^{3}H]norepinephrine uptake but competitively inhibits the binding of small molecule inhibitors such as [^{3}H]mazindol (Bryan-Lluka et al., 2003; Sharpe et al., 2001, 2003). To overcome the relatively poor chemical stability of Mr1A in solution, Xen2174 was developed. In the present studies, we used the highly selective, chemically stable, NET inhibitor Xen2174, to confirm the importance of the bulbospinal noradrenergic projection in neuropathic pain and to determine the efficacy, potency and concomitant side-effect profiles of Xen2174, relative to those of Mr1A and morphine when administered intrathecally in two different rat models of neuropathic pain.

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (225–325 g) used for the experiments involving induction of a chronic constriction injury of the sciatic nerve (CCI-rats) were purchased from the Herston Medical Research Centre, The University of Queensland (Brisbane, Australia), whereas experiments involving rats with nerve injury induced by spinal nerve-ligation were purchased from Harlan Industries (Indianapolis, USA). Rats were housed in a temperature controlled environment (21 ± 2 °C, Australia; 18–27 °C, USA) with a 12 h/12 h light/dark cycle. Food and water were available ad libitum. Ethical approval for this study was obtained from the Animal Experimentation Ethics Committee of The University of Queensland or from the Institutional Animal Care and Use Committee of the University of California, San Diego.

2.2. Reagents and materials

Aqueous stock solutions of Xen2174 (10 mg/mL; Batch # 0241A), Mr1A (10 mg/mL) and vehicle (5 mM sodium acetate in saline, pH 5.5 were supplied by the staff of Xenome Ltd (Brisbane, Australia). Morphine hydrochloride powder (BP) was purchased from Macfarlan Smith Ltd (Edinburgh, UK) and yohimbine hydrochloride powder was purchased from Tocris Cookson Ltd (Bristol, UK). Ketamine, xylazine, enrofloxacin and bupivacaine injection vials were purchased from Provet Qld Pty Ltd (Brisbane, Australia). Sodium benzylpenicillin vials (600 mg) were purchased from CSL Ltd (Melbourne, Australia) and normal saline ampoules were obtained from Delta West Pty Ltd (Perth, Australia). Nisoxetine was obtained from Sigma Aldrich (Sydney, Australia) with *l*-[7-^{3}H]-norepinephrine (specific activity, 14.9 Ci/mmol) and [^{3}H]nisoxetine (specific activity, 80 Ci/mmol) obtained from Perkin–Elmer Life Sciences Pty Ltd (Melbourne, Australia). [^{3}H]-WIN35,428, [^{3}H]-citalopram, GBR12935 and imipramine were purchased from Perkin–Elmer Pty Ltd (Melbourne, Australia). COS-1 and -7 cells were purchased from the American Type Culture Collection. Human NET (hNET) was obtained from David Kaye of the Baker Medical Research Institute (Melbourne, Australia). Rat NET (rNET) was obtained from A/Prof Lesley Bryan-LLuka, The University of Queensland (Brisbane, Australia). CHO-K1 cell membranes containing hDAT and hSERT were purchased from Perkin–Elmer Pty Ltd (Melbourne, Australia). Single lumen polyethylene tubing (ID 0.2 mm, OD 0.6 mm) was purchased from Auburn Plastics and Engineering Pty Ltd (Sydney, Australia) and sterile siliconised silk sutures (Dysilk™) were obtained from Dynek Pty Ltd (Adelaide, Australia).

2.3. Surgery

2.3.1. Chronic constriction injury (CCI) of the sciatic nerve

Rats were anaesthetised with ketamine (80 mg/kg) and xylazine (8 mg/kg) administered by intraperitoneal (i.p.) injection, and a chronic constriction injury (CCI) of the sciatic nerve was produced according to the method of Bennett and Xie (1988). Briefly, the left common sciatic nerve was exposed at mid-thigh level by blunt dissection through the *biceps femoris*. Proximal to the trifurcation, ≈10 mm of nerve was freed of adhering tissue and four loose ligatures (3.0 silk) were tied around the sciatic nerve (≈1 mm apart). In sham-operated control rats, an identical dissection was performed on the left side such that the sciatic nerve was freed from surrounding tissue, but was not ligated. The incision was closed in layers. After surgery, rats received an intramuscular (i.m.) injection of benzylpenicillin (60 mg) to prevent infection and were kept warm during surgical recovery. Rats were housed singly prior to intrathecal (i.t.) or epidural catheter insertion and until they were administered either opioid or vehicle at 14 days post-CCI-surgery.

Rats were inspected daily from the time of CCI-surgery with regard to posture of the affected hindpaw, exploring behaviour, body weight and water intake, and signs of autotomy. On rare occasions, early signs of autotomy were seen (gnawing of claw tips and some surrounding tissue on the injured hindpaw) which resulted in prompt euthanasia.

2.3.2. Spinal nerve-ligation

The surgical procedure previously described by Kim and Chung (1992) was performed to induce mechanical allodynia. Briefly, the left L-5 and L-6 spinal nerves were isolated adjacent to the vertebral column and ligated with 6-0 silk suture distal to the dorsal root ganglion under isoflurane anaesthesia. The rats were allowed a 7-day post-operative recovery period before inclusion in the study.

2.3.3. Intrathecal or epidural catheter insertion in CCI-rats

Eleven to 12 days post CCI-surgery, rats were deeply anaesthetised with a mixture of ketamine (80 mg/kg i.p.) and xylazine (8 mg/kg i.p.). Prior to surgery, the back and neck regions of the rat were shaved and the skin cleansed with antiseptic surgical scrub. The rat was then placed in a prone position and the L6 lumbar vertebra was located by palpation of the tuber sacrales of the os ileum. A 6 cm incision was made in the midline of the back, 3 cm caudal and 3 cm cephalad to L6 and a subcutaneous (s.c.) pocket (for the i.t. catheter) was formed by blunt dissection with scissors on both sides of the incision. The fascia covering the superficial muscles of the back were cut in a 5 mm V-shaped incision that encompassed L5. Additional 5 mm caudal incisions were made parallel to L6. The fascia was then retracted and the lumbar muscles surrounding the base of L5 and L6 were removed, as was the *m. interspinalis* between the spinous processes of L5–L6. Following removal of the L6 spinous processes with rongeurs, the soft tissue beneath the L5 iliac arch was removed, exposing the dura mater.

For i.t. catheter placement, the dural membrane was exposed and then incised with a 23G needle, releasing clear cerebrospinal fluid. A polyethylene catheter (OD 0.6 mm, ID 0.2 mm, 20 cm) pre-filled with saline, was carefully advanced a distance of 1 cm into the intrathecal space and a small volume of saline (20 μL) was administered through the catheter. If leakage of saline around the catheter was observed, the rat was excluded from further experimentation. By comparison, for epidural catheter placement, the polypropylene catheter (pre-filled with saline) was inserted 1 cm into the epidural space between the dural membrane and the L5 lumbar vertebra. A small volume (20 μL) of saline was administered through the epidural catheter and if saline leakage was observed, the rat was excluded from further experimentation. After successful completion of the 'leak test', the i.t. or epidural catheter was fixed with dental cement onto the surrounding muscle ≈2 cm from L5, exteriorised through a s.c. tunnel to a small incision at the base of the neck and sutured in position. After suturing of the lumbar muscles and skin, rats received benzylpenicillin (60 mg i.m.) and enrofloxacin (5 mg/kg s.c.) to prevent infection and were kept warm during recovery from anaesthesia. Following completion of the surgery, rats were housed singly for a recovery period of 2–3 days prior to i.t. or epidural drug administration.

2.3.4. Verification of correct catheter placement in CCI-rats

On the day following i.t. surgery in CCI-rats, the local anaesthetic, lignocaine (2%, 20 μL) was administered via the i.t. catheter, followed by a saline flush injection (20 μL). If complete paralysis of both hind legs was not observed, rats were excluded from further experimentation. At the completion of each experiment, malachite green dye (30 μL) was injected via the i.t. or the epidural catheter whilst rats were lightly anaesthetised with O_2:CO_2 (50%:50%). Thirty seconds later, rats were decapitated and the spinal column was exposed surgically. Data from rats, where there was evidence of dye leakage at the site where the catheter entered the back muscles above L6 or failure of the dye to distribute at least 3–4 cm rostrally, were excluded from the analysis.

2.3.5. Intrathecal catheter insertion in l5/l6 spinal nerve-ligated rats

At 7 days post-L5/L6 spinal nerve-ligation, rats were anaesthetised with isoflurane, the dura exposed and incised and an i.t. catheter (stretched PE-10) inserted 8.5 cm to the lumbar region of the spinal column and exteriorised on the top of the head as previously described (Yaksh and Rudy, 1976). A recovery interval of 4–5 days was allowed before testing was performed. At the end of the experiment, rats were killed and the spinal cords were examined. Invariably, the catheter tips were found to be correctly positioned at the L1/2 spinal level.

2.4. Assessment of anti-allodynic responses using von Frey filaments

2.4.1. CCI-rats

At 14-days post-CCI-surgery, tactile allodynia, the distinguishing feature of neuropathic pain, was quantified using von Frey filaments. CCI-rats, with an i.t. (or epidural) catheter inserted, were transferred individually to wire mesh testing cages (20 cm × 20 cm × 20 cm) and allowed to acclimatise for 10 min. Von Frey filaments were used to determine the lowest mechanical threshold required for a brisk paw withdrawal reflex (i.e. the paw withdrawal threshold; PWT). Briefly, starting with the von Frey filament that produced the lowest force, the filament was applied to the plantar surface of the hindpaw until the filament buckled slightly. Absence of a response after ≈5 s prompted use of the next filament of increasing weight. Filaments used produced a buckling weight of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 g and these were calibrated regularly. A score of 20 g was given to animals that did not respond to any of the von Frey filaments. Von Frey testing was performed at the following times: pre-dose, 0.08, 0.25, 0.5, 0.45, 1, 1.5, 2 and 3 h post-dosing. In many experiments, von Frey testing was continued beyond 3 h until baseline PWT responses were obtained at the following additional times, 4, 6, 24, 30, 48 54 and 72 h post-dosing.

2.4.2. L5/L6 nerve-ligated rats

For L5/L6 spinal nerve-ligated rats, tactile allodynia was quantified using eight von Frey filaments, calibrated in the range 0.4–15.1 g. These von Frey filaments were applied to the mid-plantar surface of the hindpaw for ≈6–8 s until a response was noted as a sharp paw withdrawal or flinching immediately upon removal of the filament. von Frey testing was performed prior to drug administration and at the following post-dosing times: pre-dose, 0.25, 0.5, 1, 2, 4 and 6 h. If a minimum stimulus was reached and positive responses still occurred, the threshold was assigned an arbitrary minimum value of 0.25 g; if a maximum stimulus was presented and no response occurred, a maximum threshold value of 15 g was assigned (Chaplan et al., 1994).

2.5. Side effects

In CCI-rats, side-effects including excessive staring, grooming and exploring, apnoea, myoclonus, head/body twitches, wet-dog-shakes, chewing, sedation and serpentine tail

movements were scored in a semi-quantitative manner according to their intensity (none; mild; moderate; severe) and nature (absent; intermittent; continuous) immediately following von Frey testing at each assessment time.

For spinal nerve-ligated rats, general behavioural assessments were made during each period of observation. Observations included: tactile allodynia (vocalisation/agitation induced by light touch applied to the body surface), spontaneous vocalisation, biting and chewing of body surface, loss of hind limb placing and stepping reflex, loss of hind limb weight bearing, and loss of righting reflex.

2.6. Storage of stock solutions of peptides

Aliquots (10 μL) of the stock solutions of each of Xen2174, Mr1A, morphine and yohimbine were stored at −20 °C prior to use for animal experimentation. Immediately prior to experimentation, individual aliquots of Xen2174 were thawed on ice and then diluted to the required concentration with vehicle (5 mM sodium acetate, pH 5.5) to produce the desired final concentration for subsequent i.t. (or epidural) administration. Solutions of Mr1A, morphine and yohimbine were diluted with isotonic saline. Unused portions of thawed stock solutions were discarded to waste, to ensure that the peptides underwent only a single freeze-thaw cycle.

2.7. Assay of Xen2174 stock solution

Stock solutions (10 mg/mL) of Xen2174 and Mr1A were assayed using high performance liquid chromatography by staff members at Xenome Ltd and verified to contain the nominal concentration.

2.8. Drug administration

2.8.1. Intrathecal administration of Xen2174, Mr1A and morphine in CCI-rats

On day 14 post-CCI surgery, individual groups of drug-naive-CCI rats received one i.t. bolus injection (10 μL) of Xen2174 (0.2, 1, 10, 20, 30 nmol; $n=6$), Mr1A (1, 5, 10 nmol; $n=4$) or morphine (3.5, 10, 17, 35, 50 nmol; $n=6$) via the surgically implanted i.t. catheter. Drug-naive, control CCI-rats ($n=4$) received bolus i.t. injections (10 μL) of vehicle or saline on day 14 post-CCI surgery. All i.t. injections in CCI-rats were followed by an i.t. saline flush injection (20 μL) to ensure complete drug delivery before von Frey testing was performed.

2.8.2. Intrathecal administration of Xen2174 in spinal nerve-ligated rats

Groups of L5/L6 spinal nerve-ligated rats that had developed neuropathy were administered one i.t. bolus injection of Xen2174 (0.7, 2 or 7; $n=6$, or 71 nmol; $n=4$) in a volume of 10 μL, followed by a saline flush (10 μL) via the surgically implanted i.t. catheter. Similarly, drug-naive, control spinal nerve-ligated rats ($n=6$) also received bolus i.t. injections of saline (10 μL). Following completion of von Frey testing, rats were killed by i.p. injection of 0.5 mL of the euthanasia solution Beuthanasia-D, or by asphyxiation in a CO_2 chamber.

2.8.3. Control studies: intrathecal Xen2174 in control non-injured and sham-CCI rats

Drug-naive, control, non-injured rats received an i.t. bolus injection (10 μL) of Xen2174 (10 nmol; $n=6$) or vehicle ($n=4$). On day 14 post-sham-CCI surgery, individual groups of drug-naive sham-CCI rats also received either an i.t. bolus injection (10 μL) of Xen2174 (10 nmol; $n=6$) or vehicle ($n=4$).

2.8.4. Epidural Xen2174 in CCI-rats

On day 14 post-CCI-surgery, individual groups of drug-naive CCI-rats received an epidural bolus injection (10 μL) of Xen2174 (30 nmol; $n=6$) or vehicle ($n=4$) followed by a saline flush injection (20 μL) via the surgically implanted epidural catheter.

2.8.5. Combined intrathecal administration of Xen2174 with yohimbine

On day 14 post-CCI surgery, a group of rats ($n=5$) received a combined i.t. bolus injection of Xen2174 (10 nmol) and the selective α_2-adrenoceptor antagonist, yohimbine (100 nmol), in a volume of 20 μL.

2.8.6. Inhibition of cellular uptake of [^{3}H]-norepinephrine and inhibition of radioligand binding to NET, DAT and SERT

Inhibition of the cellular uptake of norepinephrine subsequent to inhibition of the NET by Mr1A and Xen2174 was determined in COS-1 and -7 cells transfected with the human NET (hNET) or the rat NET (rNET) according to the method by Sharpe et al. (2003). Briefly, transiently transfected COS-1 cells were pre-incubated in the presence and absence of Xen2174 (1 μM) or Mr1A (μM) for 15 min before the addition of 100 nM [^{3}H]-norepinephrine (supplemented with unlabeled substrate as required) for 15 min. The solution containing free [^{3}H]-norepinephrine (NE) was then rapidly removed, and the cells were washed three times with ice-cold phosphate-buffered saline and lysed, before the level of radioactivity of the cell lysate was determined by liquid scintillation counting.

For radioligand binding studies at NET, membranes from transfected COS-7 cells were incubated in 96-well plates with [^{3}H]-nisoxetine (4.3 nM) in the absence or presence of Mr1A or Xen2174 (1 nM–100 μM, in triplicate) in buffer for 1 h at room temperature, as previously described (Sharpe et al., 2003). Filter-retained radioactivity was quantified by liquid scintillation counting. For radioligand binding studies at DAT, CHO-K1 cell membrane containing hDAT were incubated with the selective DAT inhibitor, [^{3}H]-WIN35,428 (30 nM), in 50 mM Tris–HCl and 100 mMNaCl at pH 7.4, together with varying concentrations of χ-Mr1A and Xen2174 (10^{-4}–10^{-11} M) or reference unlabelled selective DAT inhibitor (GBR12935). For radioligand binding studies at SERT, CHO-K1 cell membranes containing hSERT were incubated with the selective SERT inhibitor, [^{3}H]-citalopram (4 nM), in 50 mM Tris–HCl, 300 mMNaCl and 5 mMKCl at pH 7.4, together with varying concentrations of χ-Mr1A and Xen2174 (10^{-4}–10^{-11} M) or reference unlabelled ligand (imipramine, a TCA). Assays were incubated for 2 h at 4 °C for DAT, and 1 h at room temperature for SERT, and membranes filtered and counted as described for NET (Sharpe et al., 2003).

2.9. Data analysis

Paw withdrawal thresholds (PWTs; g) were normalised by subtraction of the mean individual baseline PWT values quantified immediately prior to drug administration. The area under the normalised PWT versus time curve (PWT AUC) was calculated using the trapezoidal rule. Dose–response curves were constructed by plotting the extent and duration of the normalised ipsilateral (anti-allodynic + antinociceptive) and contralateral (antinociceptive) responses (area under the normalised PWT versus time curve; PWT AUC) truncated to the first 6 h post-dosing interval, versus the i.t. dose for each of Xen2174 and morphine. ED_{50} values were estimated using non-linear regression of: (i) peak PWTs versus log dose and (ii) normalised PWT AUC values versus log dose (GraphPad Prism 3.0™, San Diego, CA).

For the cellular uptake studies, specific [^{3}H]NE uptake was the difference between uptake in the absence (total uptake) and presence (non-specific uptake) of nisoxetine. IC_{50} values for the inhibition of [^{3}H]NE uptake by each of Xen2174 and Mr1A and the associated Hill slopes for the inhibition curves were estimated using non-linear regression (GraphPad Prism 3.0™, San Diego, CA). The radioligand binding data were analysed by non-linear regression using individual data points with GraphPad Prism 3.0™. The equation of Cheng and Prusoff (1973) was used to convert IC_{50} values for [^{3}H]-nisoxetine displacement to pK_i values.

2.10. Statistical analysis

The Mann–Whitney test was used to compare differences in the normalised PWT AUC values between treatment groups. Hill slopes were compared with a slope of 1.0 using the Student's *t*-test and the significance of differences between values was determined using ANOVA followed by Dunnett's multiple comparison tests on absolute or log data, as appropriate. Statistical analyses were undertaken using the GraphPad Prism™ software package with a statistical significance criterion of $P<0.05$.

3. Results

Consistent with previous studies in the literature, tactile (mechanical) allodynia, the distinguishing feature of neuropathic pain, developed in rats following induction of either a chronic constriction injury (CCI) of the sciatic nerve (Bennett and Xie, 1988) or tight ligation of the L5/L6 spinal nerves (Kim and Chung, 1992). Specifically for CCI-rats, the mean (±SEM) ipsilateral (injured) paw withdrawal threshold (PWT) decreased significantly ($P<0.05$) from a pre-surgery baseline value of 12.1 (±0.1)–4.2 (±0.2) g at 14 days post CCI-surgery. By contrast, the corresponding mean (±SEM) contralateral (non-injured) PWT values prior to CCI-surgery (12.2±0.2 g) did not differ significantly ($P>0.05$) from that at 14 days post CCI-surgery (12.8 ± 0.2 g). For L5/L6 nerve-ligated rats, the mean (± SEM) ipsilateral PWT also decreased significantly ($P<0.05$) from a pre-surgery baseline value of 15–2.7 (±0.1) g just prior to i.t. drug administration.

3.1. I.t. Xen2174 in CCI-rats

I.t. administration of Xen2174 to CCI-rats produced a significant ($P<0.05$) dose-dependent increase in the PWT in both the ipsilateral and contralateral hindpaws (Figs. 1A–C and 2A–B). Specifically, administration of i.t. Xen2174 in a dose of 0.2 nmol to CCI-rats resulted in peak anti-allodynic and antinociceptive effects at 15 min post-dosing and a duration of action of ≈2–3 h in both the ipsilateral and contralateral hindpaws (Fig. 1A–B). When the i.t. dose of Xen2174 was increased to 1 and 10 nmol, there was a rapid increase in the PWT in both the ipsilateral and contralateral hindpaws such that the peak anti-allodynic and antinociceptive effects, respectively, occurred at 1–1.5 h and the duration of action was ≈4 h (Fig. 1A–B). Increasing the magnitude of the i.t. dose of Xen2174 further to 20 and 30 nmol again produced a rapid onset of anti-allodynic and antinociceptive actions in the ipsilateral and contralateral hindpaws, respectively, such that the mean (± SEM) PWT more than doubled by 5 min post-dosing in the ipsilateral hindpaw, with the peak effect occurring at 1–1.5 h. Based on the peak responses evoked by individual doses of i.t. Xen2174, the mean (±SEM) ED_{50} for the alleviation of tactile allodynia in the ipsilateral hindpaw was 15.7 (±3.9) nmol. The corresponding mean (±SEM) ED_{50} for antinociception in the contralateral hindpaw was estimated to be 15.2 (±2.7 nmol).

Interestingly, the durations of the anti-allodynic responses evoked by the 20 and 30 nmol doses of i.t. Xen2174 were ≈30 and ≈54 h, >7- and 13-fold longer than that observed following administration of the 10 nmol dose (Fig. 1A–C). When estimated using the PWT AUC values for the 6 h post-dosing interval, the mean (±SEM) ED_{50} for the alleviation of tactile allodynia in the ipsilateral hindpaw was 14.8 (±1.1) nmol and that for the production of antinociception in the contralateral hindpaw was 14.9 (± 1.1) nmol (Fig. 2A–B).

3.2. I.t. Xen2174 in L5/L6 spinal nerve-ligated rats

Intrathecally administered Xen2174 in doses ranging from 0.7 to 7 nmol also produced a dose-dependent reversal of tactile allodynia in L5/L6 spinal nerve-ligated rats (Fig. 3). Specifically, administration of i.t. Xen2174 in a dose of 0.7 nmol produced minimal relief of tactile allodynia such that the post-dosing PWT values were similar to both pre-dosing PWT values and the PWT values seen after i.t. injection of saline. When the dose of i.t. Xen2174 was increased to 2 nmol, there was a rapid onset of the anti-allodynic action with the peak effect observed at 1 h post-dosing and a duration of action of ≈2 h. Further increasing the dose of Xen2174 from 2 to 7 nmol resulted in a rapid onset of a significant ($P=0.001$) anti-allodynic effect with the mean peak anti-allodynic response (≈13 g PWT) produced at ≈0.5 h post-dosing and a duration of action of ≈3 h. When the magnitude of the i.t. dose of

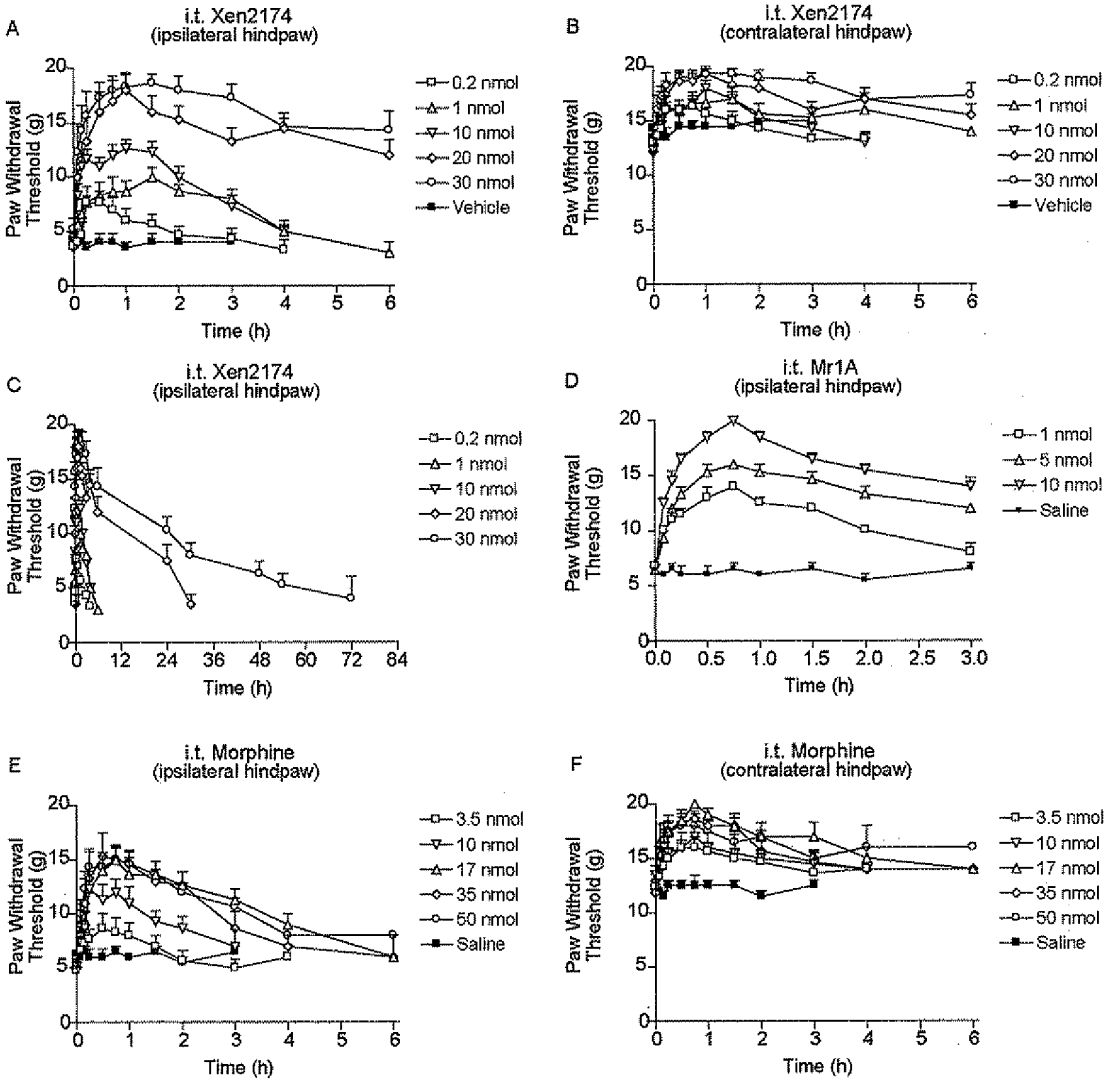

Fig. 1. The mean (±SEM) paw withdrawal threshold (PWT) versus time curves produced by bolus doses of i.t. Xen2174 ($n=6$ per dose) or vehicle ($n=4$) in CCI-rats for the: (A) ipsilateral, (B) contralateral and (C) ipsilateral hindpaws (0–72 h), (D) i.t. Mr1A ($n=4$ per dose) produced dose-dependent anti-allodynic effects in the ipsilateral hindpaw, whereas saline ($n=4$) had no significant effect, as expected. (E) Mean (±SEM) PWT versus time curves evoked by i.t. morphine ($n=6$ per dose) or saline ($n=4$) in the ipsilateral and (F) contralateral hindpaws of CCI-rats.

Xen2174 was increased 10-fold to 71 nmol, the magnitude of the anti-allodynic responses were similar to that produced by the 7 nmol dose, although the larger dose produced a faster onset of the mean peak anti-allodynic response (≈0.25 h) but a shorter duration of action (≈2 h; Fig. 3).

3.3. I.t. Mr1A in CCI-rats

Bolus i.t. doses of Mr1A (1, 5, 10 nmol) produced dose-dependent relief of tactile allodynia in the ipsilateral hindpaw of CCI-rats and dose-dependent antinociception in the contralateral hindpaw in a manner similar to Xen2174 (Fig. 1D). The peak anti-allodynic responses were observed at 0.75 h post-dosing and the durations of action were ≥3 h.

3.4. I.t. Morphine in CCI-rats

Following i.t. administration of morphine to CCI-rats, there was a rapid onset of action with the peak anti-allodynic and antinociceptive responses in the ipsilateral and contralateral hindpaws, respectively, occurring at 0.5–0.75 h post-dosing and a duration of action of up to 4 h (Fig. 1E and F). For i.t. morphine doses in the range 3.5–17 nmol, the magnitude of the anti-allodynic and the antinociceptive responses increased in a dose-dependent manner in the ipsilateral and contralateral hindpaws, respectively, similar to the responses evoked by i.t. Xen2174. However, further escalation of the i.t. morphine dose to 35 and 50 nmol revealed a pronounced ceiling effect such that the magnitude of the anti-allodynic

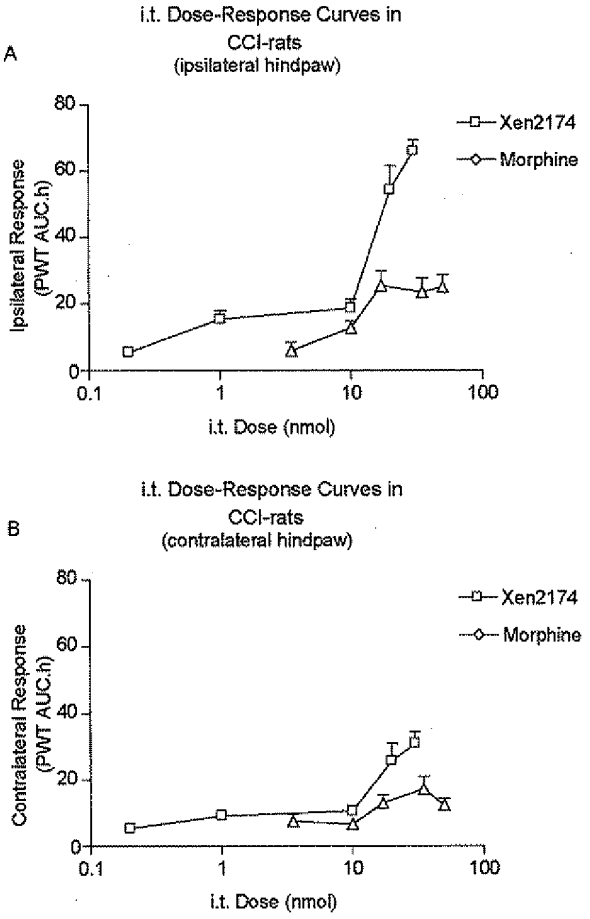

Fig. 2. Mean (±SEM) dose–response curves produced by i.t. bolus doses of Xen2174 ($n=30$) and morphine ($n=30$) for the: (A) ipsilateral and (B) contralateral hindpaws in CCI-rats.

and antinociceptive responses remained sub-maximal and did not increase beyond that produced by the 17 nmol dose (Figs. 1E,F and 2A–B). When estimated using the peak responses, the mean (±SEM) ED_{50} doses for i.t. morphine

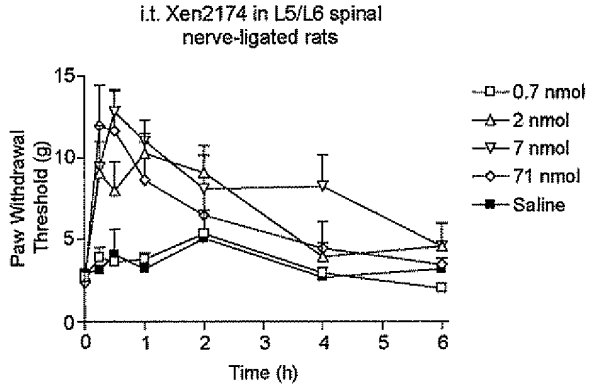

Fig. 3. The mean (±SEM) paw withdrawal threshold versus time curves for the relief of tactile allodynia produced by i.t. bolus doses of Xen2174 ($n=6$ per dose except 71 nmol ($n=4$) or saline ($n=6$)) in L5/L6 spinal nerve-ligated rats.

for alleviating tactile allodynia in the ipsilateral hindpaw and for producing antinociception in the contralateral hindpaw were 7.0 (±1.7) and 10.7 (±5.1) nmol, respectively. When estimated using the PWT AUC values, the corresponding ED_{50} s were 10.2 (±1.0) and 14.3 (±1.2) nmol, respectively (Fig. 2A and B).

3.5. I.t. Vehicle and i.t. saline

Single bolus i.t. injections of vehicle (5.5 mM sodium acetate buffer, pH 5.5) or saline did not significantly ($P>0.05$) alter PWTs in CCI-rats (Fig. 1; Table 1). Similarly, i.t. bolus injections of saline to spinal nerve-ligated rats did not produce any significant anti-allodynic response (mean ± SEM PWT AUC = 5.0 ± 4.0 PWT AUC.h; Fig. 3). These observations show that neither the vehicle, saline nor the experimental procedures themselves contributed to the anti-allodynic or the antinociceptive effects evoked by single bolus doses of i.t. Xen2174, morphine or Mr1A.

3.6. I.t. Xen2174 in control (non-injured) rats

The mean (±SEM) pre-dosing von Frey PWTs in each of the left (11.4 ± 0.3 g) and right (11.9 ± 0.4 g) hindpaws of control (non-injured) rats did not significantly differ ($P>0.05$), as expected for the non-injured state (Fig. 4A). I.t. administration of Xen2174 (10 nmol) produced significant ($P<0.05$) levels of antinociception in both hindpaws of control non-injured rats, compared with the responses evoked by i.t. vehicle (Fig. 4A; Table 1). Antinociception produced by i.t. Xen2174 peaked at ≈0.75 h with a corresponding duration of action of 4–6 h. I.t. administration of vehicle (10 μL) produced insignificant ($P>0.05$) antinociception in both hindpaws of control non-injured rats (Table 1).

3.7. I.t. Xen2174 in sham-CCI rats

The mean (±SEM) pre-dosing von Frey PWTs did not significantly differ ($P>0.05$) between the ipsilateral

Table 1
Area under the response versus time curves (PWT AUCs) in CCI, sham-CCI and control (non-injured) rats administered either i.t. Xen2174 (10 nmol) or i.t. vehicle (sodium acetate buffer)

Compound administered	Rat hindpaw	Mean (±SEM) PWT AUC (PWT AUC.h)		
		CCI-rats	Sham-CCI rats	Control non-injured rats
i.t Xen2174 (10 nmol)	Ipsilateral/left	18.7 ± 2.8	8.3 ± 1.6	8.5 ± 2.2
	Contralateral/right	10.7 ± 1.7	7.1 ± 2.9	9.8 ± 2.1
i.t. Vehicle	Ipsilateral/left	−0.8 ± 0.5	−5.4 ± 0.7	−3.5 ± 2.6
	Contralateral/right	−0.8 ± 1.3	0.1 ± 2.1	0.6 ± 3.3

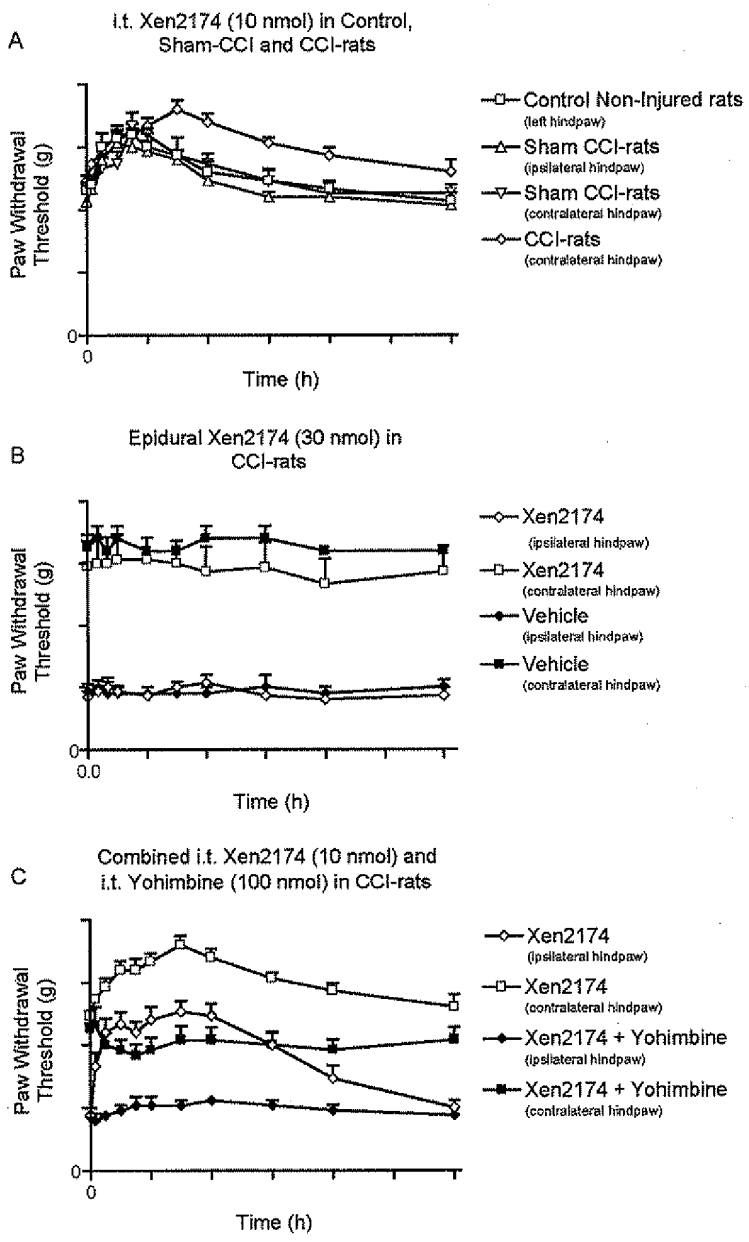

Fig. 4. The mean (±SEM) paw withdrawal threshold versus time curves produced by administration of (A) i.t. Xen2174 (10 nmol) in control non-injured ($n=6$), sham-CCI ($n=6$) and CCI rats (contralateral hindpaw, $n=6$), (B) epidural Xen2174 (30 nmol, $n=6$) and saline ($n=4$) in CCI-rats and (C) combined i.t. administration of Xen2174 (10 nmol) and yohimbine (100 nmol) in CCI-rats ($n=5$).

(11.2±0.3 g) and contralateral (11.4±0.3 g) hindpaws of sham-CCI rats ($P<0.05$) or from those of control non-injured animals, consistent with the lack of nerve injury on the ipsilateral side (Fig. 4A). I.t. administration of Xen2174 (10 nmol) produced significant ($P<0.05$) levels of anti-nociception in both hindpaws of sham-CCI rats, compared with the responses evoked by i.t. vehicle (Fig. 4A; Table 1). Antinociception evoked by i.t. Xen2174 peaked at ≈0.5 and 0.75 h post-dosing in each of the ipsilateral and contralateral hindpaws, respectively, thereafter decreasing in a mono-exponential manner; the corresponding duration of action was 3–4 h. I.t. administration of vehicle did not produce significant ($P>0.05$) antinociception in the hindpaw of sham-CCI rats (Table 1).

3.8. Comparison of the anti-allodynic responses of i.t. Xen2174 in control non-injured, sham-CCI and CCI-rats

The extent and duration of the antinociceptive responses (PWT AUC) evoked by i.t. Xen2174 (10 nmol) in the contralateral hindpaw of CCI-rats were not significantly different ($P>0.05$) from that of either hindpaw of control

non-injured rats or from that in the ipsilateral or contralateral hindpaw of sham-operated-CCI rats; all of these responses were significantly less than that evoked by i.t. Xen2174 (10 nmol) in the ipsilateral hindpaw of CCI-rats (Fig. 4A; Table 1).

3.9. Epidural Xen2174 in CCI-rats

Epidural administration of Xen2174 (30 nmol) was completely ineffective for the relief of tactile allodynia in the ipsilateral hindpaw of CCI-rats (mean ± SEM PWT AUC = 0.8 ± 0.8 PWT AUC.h; Fig. 4B). Similarly, there was a lack of antinociception in the contralateral (non-injured) hindpaw of CCI-rats (−1.2 ± 2.1 PWT AUC.h; Fig. 4B). As expected, epidural administration of vehicle produced insignificant anti-allodynia/antinociception in the ipsilateral and contralateral hindpaws of CCI-rats (0.1 ± 1.0 and 0.1 ± 3.8 PWT AUC.h, respectively; Fig. 4B).

3.10. Combined i.t. administration of Xen2174 with Yohimbine

Combined i.t. administration of the α_2-adrenoceptor blocker, yohimbine (100 nmol) with Xen2174 (10 nmol) abolished the pain-relieving effects of Xen2174 in both the ipsilateral and the contralateral hindpaws of CCI-rats (Fig. 4C) such that the mean (±SEM) extent and duration of the anti-allodynic and antinociceptive responses (PWT AUC values) produced in the ipsilateral and contralateral hindpaws were significantly reduced (4.0 ± 2.6 and 8.5 ± 8.5 PWT AUC.h, respectively; $P < 0.05$) compared with the PWT AUC values produced by bolus doses of i.t. Xen2174 (10 nmol) alone in CCI-rats (Table 1).

3.11. Behavioural effects observed following i.t. administration of Xen2174, Mr1A and morphine

Following i.t. administration of the lower bolus doses of Xen2174 (0.2–10 nmol) to CCI-rats, only mild, intermittent behavioural side-effects including myoclonus, increased exploring, grooming and chewing were observed for up to ≈2 h post-dosing. The two largest doses of i.t. Xen2174 administered to CCI-rats (20 and 30 nmol) produced mild staring and mild head/body shakes, with mild apnoea being observed in the interval 24–54 h in two of six rats that received 30 nmol of i.t. Xen2174. No significant behavioural deficits were observed in L5/L6 spinal nerve-ligated rats administered i.t. Xen2174 in doses of 0.7, 2 and 7 nmol. However, when the i.t. dose of Xen2174 was increased to 71 nmol, serpentine movements of the tail were observed, although no other changes in motor function were noted. The side effects produced by i.t. administration of Xen2174 (10 nmol) in control non-injured and sham-CCI-rats were very similar to those observed following i.t. Xen2174 (10 nmol) in CCI-rats. Following i.t. administration of vehicle or saline, insignificant changes in behaviour were observed. Similarly, side-effects were not observed following epidural administration of either Xen2174 (30 nmol) or vehicle, or following combined i.t. administration of bolus doses of Xen2174 and yohimbine. The behavioural sided-effects produced by i.t. Mr1A (1–10 nmol) in CCI-rats were also relatively mild and included intermittent exploring and excessive grooming behaviour. Dose-related behavioural side effects were produced by single bolus doses of i.t. morphine (3.5–50 nmol), which were generally of a greater intensity and incidence compared with those observed for i.t. Xen2174. Following i.t. administration of morphine in doses of 3.5 and 10 nmol, mild to moderate behaviours were observed including intermittent myoclonus, chewing, increased grooming and exploring. At the higher doses (17, 35, 50 nmol), moderate staring behaviour and mild apnoea were also observed.

3.11.1. Effect of Xen2174 and Mr1A on the cellular uptake of [^{3}H]-norepinephrine and on radioligand binding to NET, DAT and SERT

The uptake of [^{3}H]-norepinephrine via the hNET or rNET in COS-1 cells was sensitive to inhibition by both Xen2174 and Mr1A. The mean (±SEM) IC_{50} values for the inhibition of NE uptake were 0.7 (±0.3) nM ($n = 10$), 409 (±29) nM ($n = 10$), and 192 (±26) nM ($n = 3$) for the control ligand, nisoxetine at hNET, relative to that of Xen2174 at hNET and rNET, respectively. The mean (± SEM) reduction in NE uptake was 60 (±5) % irrespective of NE concentration. The mean (±SEM) K_d for NE uptake in the absence (2.2 ± 0.9 nM) of Xen2174 did not differ significantly ($P > 0.05$) from that determined in the presence of Xen2174 (2.8 ± 0.8 nM) ($n = 3$); similar results were obtained at each of rNET and hNET.

The binding of the NET inhibitor, [^{3}H]-nisoxetine to the membranes of COS-7 cells expressing the hNET was competitively inhibited by both Mr1A and Xen2174. The corresponding mean (±SEM) pIC_{50} values were 5.68 (± 0.07; $n = 5$) and 5.71 (±0.07; $n = 8$) for Xen2174 and Mr1A, respectively. Similarly, Xen2174 and Mr1A were found to inhibit [^{3}H]-nisoxetine binding to the rNET (data not shown). Neither Mr1A nor Xen2174 (≤100 μM) produced significant (<5%) inhibition of [^{3}H]-citalopram or [^{3}H]-WIN35,428 binding to hSERT or hDAT, respectively ($n \geq 3$ experiments).

Thus, Xen2174, like the χ-conopeptide, Mr1A, non-competitively inhibits [^{3}H]NE uptake but competitively inhibits the binding of a small molecule inhibitor, [^{3}H]-nisoxetine.

4. Discussion

This study shows that Xen2174 is a highly selective non-competitive inhibitor of the norepinephrine transporter (NET) in a manner similar to the χ-conopeptide, Mr1A (Bryan-Lluka et al., 2003; Sharpe et al., 2001, 2003).

4.1. Antihyperpathic effects of spinal χ-conopeptides: comparison with morphine

After bolus i.t. delivery, Xen2174 produced dose-dependent anti-allodynic responses in two rat models of neuropathic pain. I.t. Xen2174 also evoked dose-dependent antinociception in the hindpaws of sham-operated and control non-injured animals with a potency similar to that seen in the contralateral hindpaws of CCI-rats. In CCI-rats, the structurally related χ-conopeptide, Mr1A, produced similar responses to Xen2174, extending previous findings that i.t. Mr1A produced dose-dependent hotplate antinociception in non-injured mice (McIntosh et al., 2000).

The potency of i.t. Xen2174 in the ipsilateral and contralateral hindpaws of CCI-rats was similar but the extent and duration (PWT AUCs) of the ipsilateral responses were ~2-fold larger than the respective contralateral responses (Table 1). These findings are similar to the significantly larger thermal and/or mechanical anti-hyperalgesic effects of bolus doses of i.t. clonidine in L5/L6 spinal nerve-injured rats compared with the respective responses produced in non-injured animals (Paqueron et al., 2003; Poree et al., 1998).

Although i.t. morphine was more potent than i.t. Xen2174 for the relief of tactile allodynia in CCI-rats, the duration of action of i.t. Xen2174 (30 nmol) was ~10-fold longer than that for a similarly large dose of i.t. morphine (35 nmol), in agreement with the observation that the duration of anti-allodynia produced by low-dose i.t. Xen2174 (1 nmol) was similar to that produced by a 10-fold larger dose of i.t. morphine (10 nmol). These preclinical findings suggest that i.t. Xen2174 may have a relatively long duration of action for the relief of moderate to severe neuropathic pain in the clinical setting.

In CCI-rats, spinal morphine produced dose-dependent anti-allodynia in a manner similar to several previous reports (Scott et al., 2002; Suzuki et al., 1999; Yu et al., 1997). However, i.t. morphine (but not i.t. Xen2174) also displayed a pronounced sub-maximal 'ceiling' effect for doses exceeding 17 nmol. Thus, although i.t. morphine appears to be more potent than i.t. Xen2174 in CCI-rats, it has a lower efficacy than i.t. Xen2174.

Interestingly, multiple studies have reported i.t. morphine to be significantly less effective or ineffective in nerve-injured versus non-injured rodents (Lee et al., 1995; Mao et al., 1995; Pertovaara and Wei, 2003; Wegert et al., 1997). Plausible mechanisms underpinning reduced efficacy of i.t. morphine in nerve-injured compared with non-injured rats, include: (i) an augmentation of descending facilitatory brainstem-spinal pathways involving NMDA receptors in the rostroventromedial medulla (Pertovaara and Wei, 2003), (ii) increased expression of anti-opioid peptides (e.g. cholecystokinin, neuropeptide-FF, dynorphin A) in the spinal cord (Nichols et al., 1995; Vanderah et al., 2000; Xu, Puke, Verge, Wiesenfeld-Hallin, Hughes and Hokfelt, 1993), and/or (iii) loss of presynaptic μ-opioid receptors or a loss of inhibitory neurones in the dorsal horn of the spinal cord secondary to nerve injury (Lee et al., 1995; Porreca, Tang, Bian, Riedl, Elde and Lai, 1998; Yaksh et al., 1995). Recently, Eisenach and Lindner (2004) also speculated that inter-laboratory differences in experimental methods and/or 'experimenter bias' may contribute to between-study differences in the apparent anti-neuropathic effectiveness of i.t. morphine (Eisenach and Lindner, 2004); further investigation is clearly warranted.

The failure of epidural Xen2174 (30 nmol) to produce significant anti-allodynic or antinociceptive effects in CCI-rats was not unexpected, as the peptide backbone of Xen2174 has insufficient lipophilicity to cross the tight junctions of the dural membrane and enter the spinal cerebrospinal fluid due to its hydrophilic nature and high capacity to form hydrogen bonds with water molecules (Burton et al., 1996; Zhao et al., 2003).

4.2. Side-effects of spinal χ-conopeptides

Although the incidence/severity of side-effects produced by i.t. Xen2174 and Mr1A in CCI-rats increased in a dose-dependent fashion, the side-effect profiles were of a mild and intermittent nature. After administration of the largest dose of Xen2174 (30 nmol) in CCI-rats, mild apnoea was observed in two of six rats in the interval 24–54 h. Interestingly, marked sedation was absent whereas this is a frequently reported side-effect following i.t. clonidine administration (Eisenach et al., 1996). Thus, clinical use of i.t. NET inhibitors may be associated with a lower incidence of adverse-effects compared with α_2-adrenoceptors agonists which produce hypotension, bradycardia, dry mouth, in addition to the risk of severe rebound systemic hypertension following abrupt cessation of long-term i.t. clonidine infusions (Eisenach et al., 1996; Hassenbusch et al., 2002; Kawamata et al., 2003).

In L5/L6 spinal nerve-ligated rats, the highest dose of Xen2174 (71 nmol) produced serpentine tail movements, similar to that produced by i.t. administration of ω-conopeptide N-type calcium channel blockers, such as MVIIA (also known as SNX-111), CVID (also known as AM336) and SNX-239 (Malmberg and Yaksh, 1995; Scott et al., 2002; Smith et al., 2002). Although, there is no known relationship between serpentine tail movements in rats and clinical responses in humans, serpentine movements may indicate spinal cord excitation.

4.3. Mechanism(s) of action of spinal χ-conopeptides in neuropathic pain: NET and α_2-adrenoceptors

As the χ-conopeptides, Mr1A and Xen2174, are highly selective, non-competitive NET inhibitors, their spinal anti-allodynic effects are likely due to enhanced activation of spinal α_2-adrenoceptors subsequent to NET inhibition. This proposal is supported by the reversal of Xen2174 anti-allodynia by spinal yohimbine (α_2-antagonist) in a dose

similar to those used with i.t. α_2-agonists previously (Takano and Yaksh, 1992), consistent with augmented terminal release of norepinephrine from bulbospinal noradrenergic pathways (Tyce and Yaksh, 1981) as well as the regulatory effects of spinally delivered α_2-agonists (Reddy et al., 1980). Previous work showing that brainstem stimulation and intracerebral morphine depress spinal nociceptive processing with complete reversal by spinal α_2-antagonists (particularly those with a preference for $\alpha_{2non\text{-}A}$ receptor subtypes) demonstrates the functional efficacy of these bulbospinal systems (Camarata and Yaksh, 1985; Takano and Yaksh, 1992; Yaksh, 1979). Additional studies showing that spinal α_2-agonists can block nociceptive processing (see Yaksh, 1985, 1999) and alleviate allodynia that occurs secondary to nerve injury in a manner reversible by spinal α_2-antagonists including yohimbine (Abelson and Hoglund, 2004; Duflo et al., 2000; Fairbanks et al., 2000; Hao et al., 1999; Kawamata et al., 2003; Paqueron et al., 2003; Yaksh et al., 1995), further support this notion.

Previous findings suggest that the antinociceptive actions of i.t. α_2-agonists such as clonidine, dexmedetomidine, ST-91 and moxonidine (Duflo et al., 2002; Fairbanks et al., 2000; Yaksh et al., 1995) are mediated by α_{2A} and/or $\alpha_{2non\text{-}A}$ adrenoceptor subtypes. It has been argued that in nerve-injured animals, these actions may be mediated predominantly by an $\alpha_{2non\text{-}A}$ subtype (Duflo et al., 2002), possibly the α_{2C}-subtype that is upregulated in the spinal cord following nerve injury (Fairbanks et al., 2000; Stone et al., 1999). In support of the view that peripheral nerve injury may induce adaptive neuroplastic changes in the central nervous system, resulting in enhanced descending noradrenergic inhibition, Ma and Eisenach (2003) recently found a consistently larger number of tyrosine hydroxylase (TH)- and dopamine-β-hydroxylase (DβH)-immunoreactive (IR) neurones in the ipsilateral locus coeruleus together with an increase in the number of TH- and DβH-IR nerve terminals in the ipsilateral lumbar dorsal horn 2 weeks after CCI-surgery in mice. Clearly, these findings together with the afore-mentioned upregulation of spinal dorsal horn α_2-adrenoceptors after nerve injury, support the notion that upregulation of descending noradrenergic inhibitory inputs to the ipsilateral spinal dorsal horn (Ma and Eisenach, 2003), may underpin, at least in part, the larger anti-allodynic/anti-hyperalgesic/antinociceptive responses evoked by intrathecally administered NET inhibitors (Xen2174 and Mr1A) or the α_2-adrenoceptor agonist, clonidine, in rodents with a CCI-injury c.f. non-injured animals.

An interesting aspect of these selective NET inhibitors is that they do not interact with catecholamine receptors and do not produce effects upon other bulbospinal projections, particularly those for serotonin and dopamine. While there is evidence that descending serotonergic systems may initiate an inhibitory effect (Bardin et al., 1997; Solomon and Gebhart, 1988; Yaksh and Wilson, 1979), comparable data indicates that increased dorsal horn serotonergic activity may be excitatory and pronociceptive, resulting in facilitation of dorsal horn nociceptive processing (Green et al., 2000; Todd and Millar, 1983; Zhou and Gebhart, 1991). Thus, any facilitation of this serotonergic component, as might be achieved by SERT or non-selective NET/SERT uptake inhibitors, could confound antinociception that would otherwise be produced by a selective augmentation of spinal noradrenergic tone.

In summary, intrathecal bolus doses of the χ-conopeptide, Xen2174, produced dose-dependent anti-allodynia in two rat models of neuropathic pain, with only mild side-effects. Collectively, in vitro and behavioural findings indicate that Xen2174 exerts its anti-allodynic effects via highly selective, non-competitive inhibition of the NET, confirming previous work showing the important modulatory role of norepinephrine in neuropathic pain. Since i.t. Xen2174 produced little antinociception against mechanical and thermal hyperalgesia in rat models of inflammatory pain (unpublished data), it appears that augmented descending inhibition via released norepinephrine may modulate allodynic pathways preferentially, thereby comprising an important adaptive inhibitory response to nerve injury. In conclusion, Xen2174 appears to be a promising drug candidate for development as a novel intrathecal drug for administration to patients with persistent neuropathic pain.

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